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Experimental Study of the Effect of a Series of Phosphoroorganic Pesticides (Dipterex and Imidan) on Embryogenesis

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Experiments conducted on pregnant Wistar rats show that chlorophos (Dipterex) has embroyotoxic and teratogenic effects after oral introduction in a 80 mg/kg dose during a critical period of embryogenesis. Embryotoxic and teratogenic effects are absent during the introduction of 8 mg/kg of the pesticide.

The oral introduction of phthalophos (Imidan) in a 30 mg/kg dose once on day 9 of pregnancy and the introduction of a 1.5 mg/kg dose daily throughout the course of pregnancy caused increased postimplantation mortality of embryos. A dose of 30 mg/kg of phthalophos on day 9 or day 13 of pregnancy causes developmental abnormalities, including hyponathia and hydrocephaly. A 0.06 mg/kg phthalophos dose does not affect the course of embryogenesis in white rats.

Thus the organophosphate pesticides Dipterex and Imidan exhibit embryotoxic and teratogenic effects at doses which significantly exceed the actual amounts of the pesticide that can enter the human organism.

Infant mortality during the past few decades has been reduced more than tenfold, childhood infections have been eradicated, have become rare, or have taken a mild form. However, the increase in birth defects and perinatal mortality has become a serious medical and social problem. On the initiative of the World Health Organization, a study was conducted a few years ago at 48 special centers located all over the world of 30 million newborn infants. The number of anatomic defects varied from 0.7 to 2%, depending on the degree of development of the country and the reliability of the reporting system. On the average, the perinatal mortality index was 25/1000 live births. It is thus important that "the child be born healthy, as well as alive."

In order to reduce the number of birth defects and to avoid a repetition of tragic consequences such as those which occurred as the result of thalidomide, it is mandatory that each new substance intended for wide-spread use, including pesticides, be studied with respect to teratogenesis.

At present, both studies which are concerned with the characteristics of the existing undesirable shifts in the environment, and those dealing with prevention of such changes are significant and of interest. The task of modern hygiene is to study the changes in the environment and to find the means for preventing or reducing particularly the teratogenic and embryotoxic effects of such changes.

The problem of teratogenic effect can be approached from the points of view of various disciplines: embryology, pharmacology, pathophysiology, and public health.

From the standpoint of public health, one must consider the difficulty of extrapolating data from experimental teratology to the human fetus. Such an extrapolation becomes feasible only after detailed analysis of the fine mechanisms of

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teratogenesis: the metabolism of a substance in the mother and in the fetus and its properties in man and in animals.

In terms of public health, those substances whose teratogenic and use levels are close—i.e., those cases where there is a great discontinuity between the teratogenic dose and a level toxic to the maternal organism—require especially careful study. In such cases, the probability of manifestation of teratogenic effects in man is great.

For substances which have a teratogenic effect at the toxic dose level (for which it is possible to establish the so-called "no-effect levels"), standards must be set for the maternal organism which take into account these data.

The cooperation of the Soviet Union and the United States on the problem of environmental health, in particular on the problem of the teratogenic effect of chemical substances, is aiding in the development and standardization of methodologies which in turn will serve to accelerate the solution of the problem.

One of the aspects of the large and complex cooperative study now in progress is an experimental study of the effect of phosphoroorganic compounds such as chlorophos (Dipterex) and phthalophos (Imidan) on embryogenesis, in conjunction with the possible ingestion of these pesticides by pregnant women with food, air, and water.

In conducting experiments to establish teratogenic potential, Wilson's serial section method (*1*) was used to detect alterations of the internal organs. The fetal skeletons were stained with Alizarin Red S to permit detection of skeletal alterations.

The experiments were conducted on pregnant Wistar rats. Chlorophos was introduced by gavage to several groups of animals simultaneously on day 9 or day 13 of pregnancy (the so-called sensitive periods in embryogenesis) with the use of a dose of 80 mg/kg (1/10 LD_{50}), or alternately daily during the course of the pregnancy using an 8 mg/kg dose (1/100 LD_{50}). All females were killed on day 19 of gestation. (Day 1 was considered to be the day sperm were found in the vaginal lavage.)

Death of the embryos before and after implanation, abnormalities in development, and fetal dimensions served as the criteria for determining fetal damage. During the course of the given experiment nearly a thousand embryos were analyzed. The experimental data obtained were processed statistically.

Administration of chlorophos at a dose of 80 mg/kg (1/10 LD_{50}) to rats on day 9 of pregnancy (Table 1) resulted in an insignificant increase in embryonic death (14.1% in comparison to 10.9% for the control group). In the group of rats given chlorophos on day 13 of pregnancy the number of normally developing fetuses dropped to 63.1% in comparison to 89.1% for the control group ($p < 0.05$), while the number of corpora lutea for the experimental and control groups was nearly the same; 11.8 and 12.5, respectively. This indicates

Table 1. Effect of chlorophos (Dipterex) on embryo development in rats.

No. of animals	Chlorophos dose (gavage)		Parameter[a]	No. of *corpora lutea*	No. of *live* fetuses	No of dead fetuses		
	Concn, mg/kg	Time or frequency				Total	Pre-*implanta*-tion	Post-*implanta*-tion
11	None (control group)		Σ	137	122	15	9	6
			$M \pm m$	12.5	11.1 ± 0.5	1.4 ± 0.5	0.8 ± 0.4	0.5 ± 0.3
			%	100.0	89.1	10.9	6.6	4.3
11	80	Single dose, day 9 of pregnancy	Σ	128	110	18	5	13
			$M \pm m$	11.6 ± 0.5	10.0 ± 0.7	1.6 ± 0.5	0.4 ± 0.2	1.1 ± 0.3
			t		0.78			1.42
			p		>0.05	>0.05	>0.05	<0.05
			%	100	85.9	14.1	3.9	10.2
11	80	Single dose, day 13 of pregnancy	Σ	130	82	48	11	37
			$M \pm m$	11.8 ± 0.67	75.6 ± 1.6	4.3 ± 0.9	1.0 ± 0.3	3.4 ± 0.7
			t		2.15	2.82		3.81
			p		<0.05	<0.02	<0.05	<0.01
10	8	Daily throughout pregnancy	Σ	117	97	20	3	17
			$M \pm m$	11.7 ± 0.4	9.6 ± 1.4	2.0 ± 1.3	0.3 ± 0.2	1.7 ± 1.3
			t		0.94			
			p		>0.05	>0.05	>0.05	>0.05
			%	100	82.8	17.2	2.5	14.7

[a] Σ = total; $M \pm m$ = mean ± S.E.; t = Student's test; p = statistical significance.

identical reproductive capacities. In addition, the mortality of embryos increased significantly after implantation; 3.4 per female in comparison to 0.5 for the control group. General edema was sharply expressed in the majority of the dead fetuses of this group (Fig. 1). Such abnormalities as exencephaly (Fig. 2) and the "nonclosing eyelid" symptom (Fig. 3) were noted in the serial sections of the fetuses which had been subjected to the effect of chlorophos on the thirteenth day of development.

FIGURE 1. General edema of a fetus after administration of chlorophos at 80 mg/kg on day 9 of pregnancy.

We could not show any significant deviation in the embryogenesis of rats in comparison to the control group after daily administration of chlorophos at 8 mg/kg/day. During the analysis of the skeletal system of embryos only isolated cases of wavy ribs were observed.

Thus chlorophos (Dipterex) was teratogenic and embryotoxic in pregnant Wistar rats at doses 2.5×10^3 times above that likely to enter the human by all routes in a 24 hr period in view of the standard previously established for chlorophos in the Soviet Union.

Turning our attention to the fact that there were no embryotoxic or teratogenic effects noted with chlorophos administration at 8 mg/kg/day (exceeding the real dose for man by 250 times), it is obviously possible to consider that chlorophos does not present an actual teratogenic danger to man with respect to its oral introduction into the organism.

In studying the embryotoxic effects of phthalaphos (Imidan), the compound was given orally (by gavage) in a single dose of 30 mg/kg (1/5 LD_{50}) to intact pregnant rats on day 9 or 13 of pregnancy; an additional group of rats were given phthalophos every other day throughout pregnancy at a dose of 1.5 mg/kg (1/100 LD_{50}), (Table 2).

Introduction of phthalophos on day 9 of pregnancy resulted in an insignificant increase in

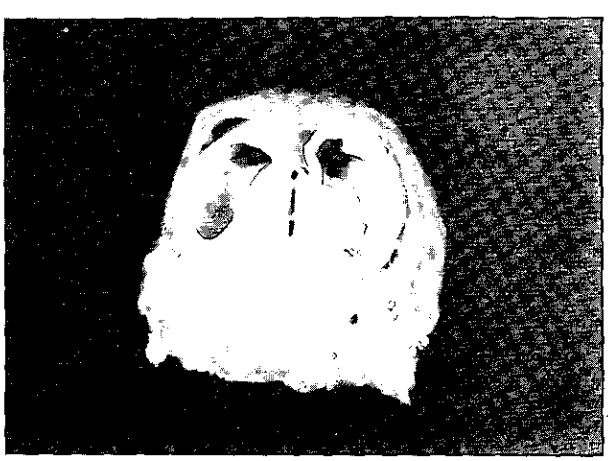

FIGURE 2. Hydrocephaly after chlorophos administration on day 13 of pregnancy.

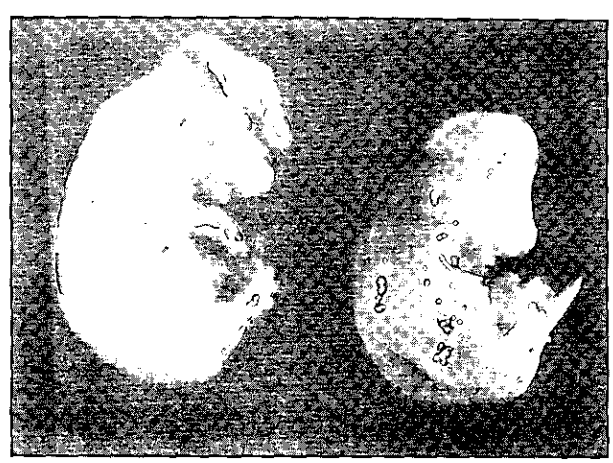

FIGURE 3. Examples of meningoencephalies seen after chlorophos administration on day 13 of pregnancy.

Table 2. Effect of phthalophos (Imidan) on embryo development in rats.

No. of animals	Phthalophos dose (gavage) Concn, mg/kg	Time or frequency	Parameter	No. of corpora lutea	No. of implantation sites	No. of live fetuses	No. of dead fetuses: Pre-implantation	No. of dead fetuses: Post-implantation
13	None (control group)		Σ	176	163	151	13	12
			$M \pm m$	13.5	12.5	12.0 ± 0.7	1.0 ± 0.25	0.9 ± 0.24
			%	100		85.7	7.39	6.82
9	30	Single dose, day 9 of pregnancy	Σ	115	105	87	10	18
			$M \pm m$	12.7	11.6	9.55 ± 1.05	1.22 ± 0.43	2.0 ± 0.5
			t			2.12	0.47	2.0
			p			<0.05	>0.1	>0.05
			%	100		75.7	8.7	15.5
8	30	Single dose, day 13 of pregnancy	Σ	110	102	92	8.0	10
			$M \pm m$	13.7	12.7	11.5 ± 1.0	1.0 ± 0.25	1.25 ± 0.25
			t			0.41		0.95
			p			>0.5	>0.5	<0.5
			%	100		84	7	9
10	None (control group)		Σ	115	103	100	12	3
			$M \pm m$	11.5	10.3	10.0 ± 0.36	1.2 ± 0.29	0.3 ± 0.1
			%	100		86.9	10.4	2.6
10	1.5	Every other day throughout pregnancy	Σ	105	88	66	17	22
			$M \pm m$	10.5	8.8	6.6 ± 0.5	1.7 ± 0.39	2.2 ± 0.89
			t			5.5	1.0	2.1
			p			<0.001	>0.25	<0.5
			%	100		62.9	16.1	21
10	0.06	Every other day throughout pregnancy	Σ	121	108	102	12	6
			$M \pm m$	12.1	10.8	10.2 ± 0.38	1.2 ± 0.3	0.6 ± 0.27
			t			0.39		1.0
			p			>0.5		>0.25
			%	100		85	10	5

postimplantation mortality of embryos and in malformations such as hypognathia (Fig. 4), general edema, and dislocation of extremities (Figs. 5 and 6).

Introduction of phthalophos on day 13 of pregnancy did not affect embryo mortality before or after implantation. However, examination of serial sections revealed hydrocephaly in 33 of the 55 embryos studied.

Introduction of the compound at a level of 1.5 mg/kg daily throughout pregnancy resulted in a statistically verifiable reduction in the number of live fetuses in the test group; 62.8% in comparison to 87% for the control group. Hydrocephaly and

FIGURE 4. Subluxation of extremities and hypognathia in a fetus after administration of phthalophos on day 9 of pregnancy.

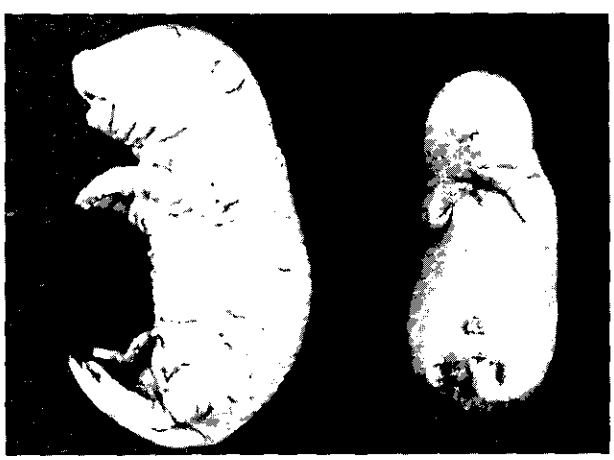

FIGURE 5. General swelling of the torso and hypognathia of a fetus after phthalophos administration on day 9 of pregnancy.

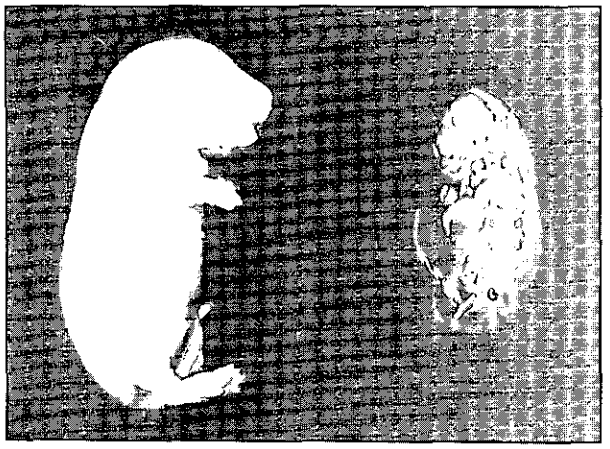

FIGURE 6. Hypognathia in a fetus with respect to the action of phthalophos on day 9 of pregnancy.

subcutaneous hemorrhages were also seen. Since phthalophos was embryotoxic, experiments were conducted to study a broad range of multiple doses, from 15 mg/kg (1/10 LD_{50}) to 0.06 mg/kg. Embryo toxicity decreased with decreased dosage; no adverse effect was noted at a dose of 0.06 mg/kg/day.

Thus phthalophos administered orally exhibited embryotoxicity and teratogenicity, but these effects were manifested at doses which significantly exceeded the actual amount of the pesticide likely to enter the human organism as it is used in the U.S.S.R.

REFERENCE

1. Wilson, J. G. Embryological considerations in teratology. In: Teratology: Principles and Techniques. J. G. Wilson and J. Warkany, Eds., University of Chicago Press, Chicago, 1965.